Supplementary Information

Unravelling the Role of Silent Mutation in ω- subunit of *E. coli* RNA polymerase: Structure Transition Inhibits Transcription

*Unnatiben Rajeshbhai Patel, Sudhanshu Gautam, Dipankar Chatterji**

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

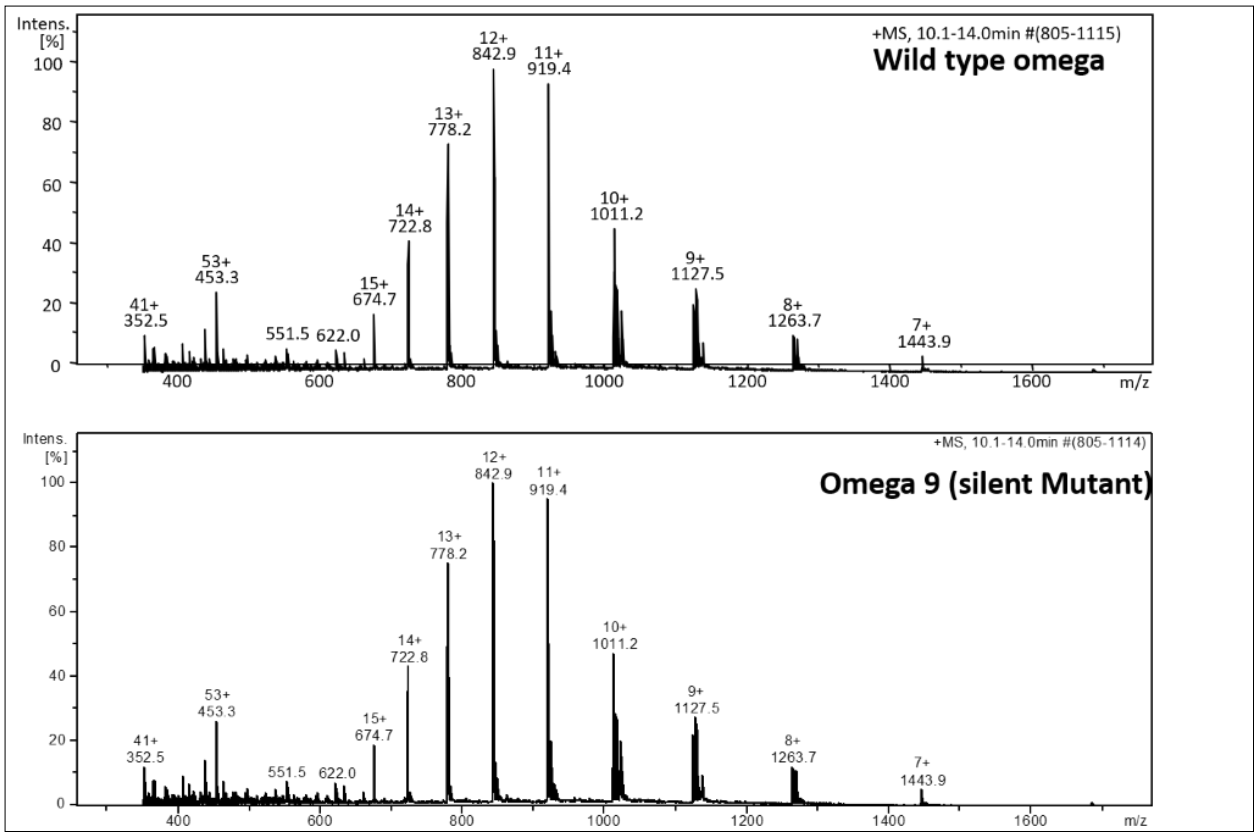


Figure S1. Mass spectroscopic analysis of wild type ω and its silent mutant ω_9. ESI analysis of protein shows that the protein sequence remains the same as wild type ω in silent mutant. The fragmentation pattern was similar for wild type and silent mutant protein.

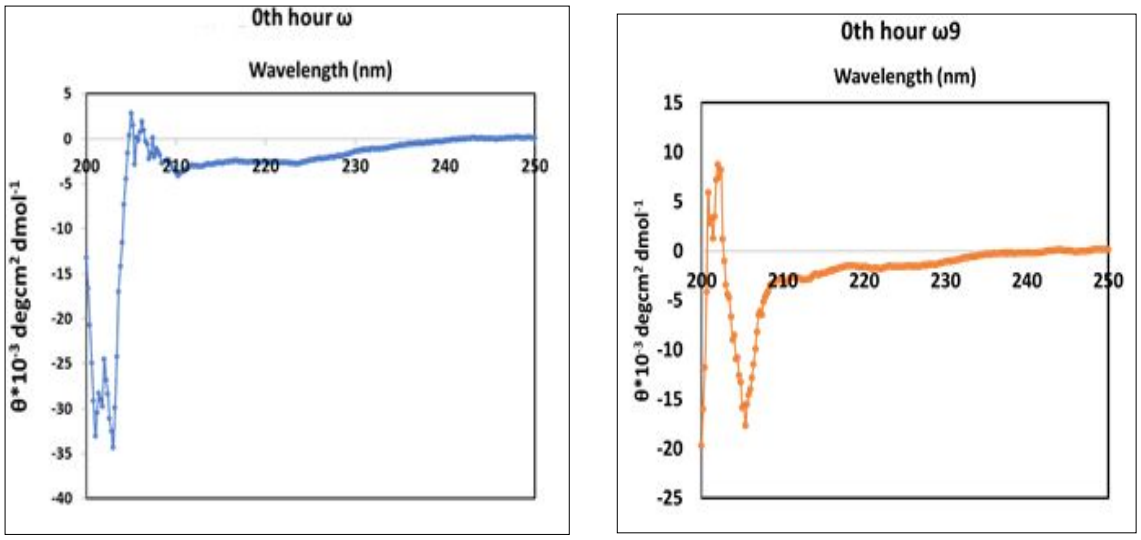
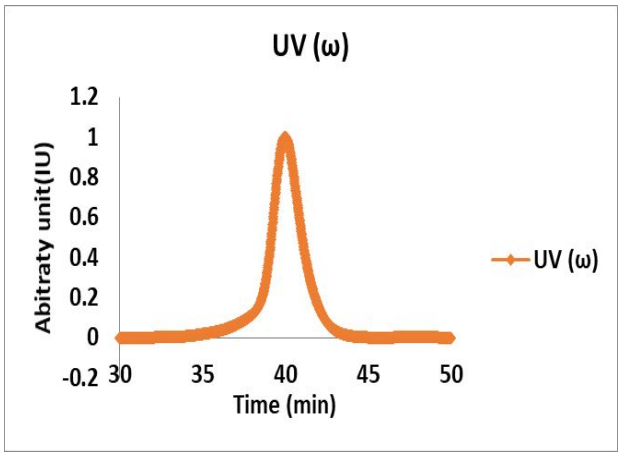


Figure S2. Refolding experiment followed for ω and ω_9. Proteins were unfolded by incubating them with 7M urea for 2 hours. CD curve shows unfolded protein at the start of refolding experiment. Protein refolding was followed by CD spectroscopy by collecting aliquots at different time interval. Curves were plotted without smoothening and presence of urea showed noisy baseline.

(A)



(B)

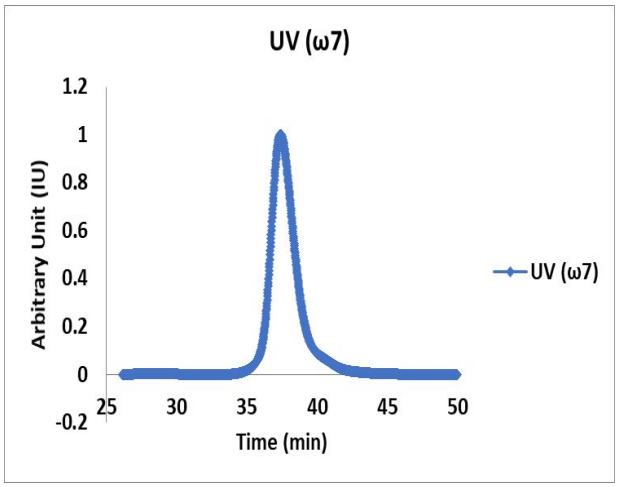
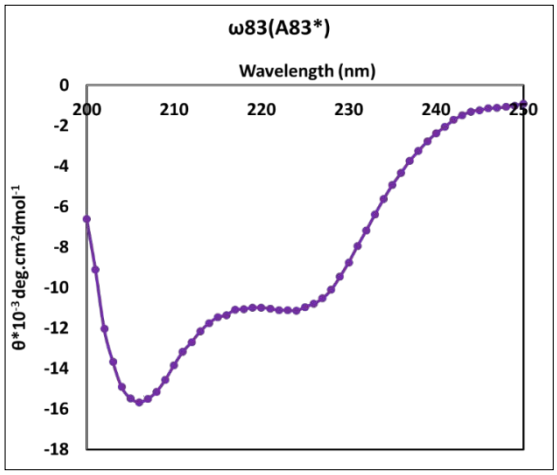


Figure S3. SEC-MALS data for ω and its silent mutant.s (A). ω (B). ω_7. Both wild type and mutant proteins exists as monomers in solution. It indicates that the protein did not form oligomers due to misfolding and CD profil is of monomeric soluble proteins. Absence of any other peak in the SEC profile of the wild type and silent mutant protein indicates that there is no aggregation of over expressed proteins.

(A)

Amino Acid	Codon	Frequency of codon usage
Valine	**GTT**	**0.29**
Valine	GTC	0.20
Valine	**GTA**	**0.17**
Valine	GTG	0.34

(B)



(C)

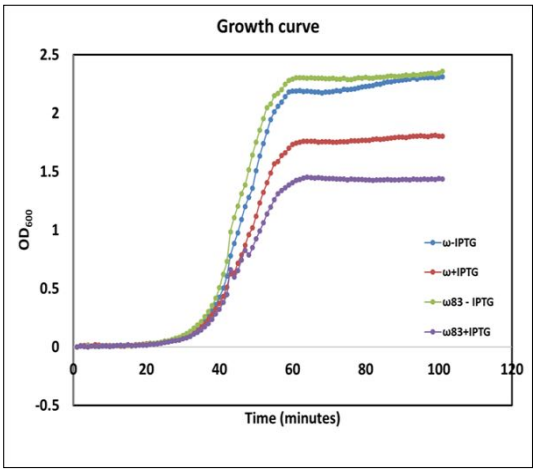
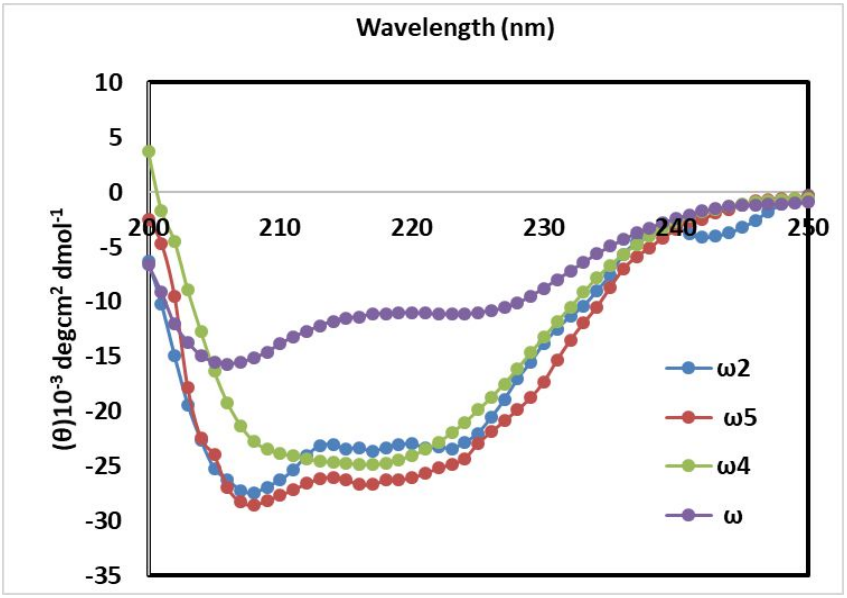


Figure S4. ω_{83} silent mutation for valine at 83rd position in *rpoZ* gene *(A)* Codon usage index for valine. *(B)* CD profile of valine silent mutant at 83rd position (GTT→GTA), frequent codon to a rare codon mutation. *(C)* Lethality check for the mutant upon induction

A)



(B)

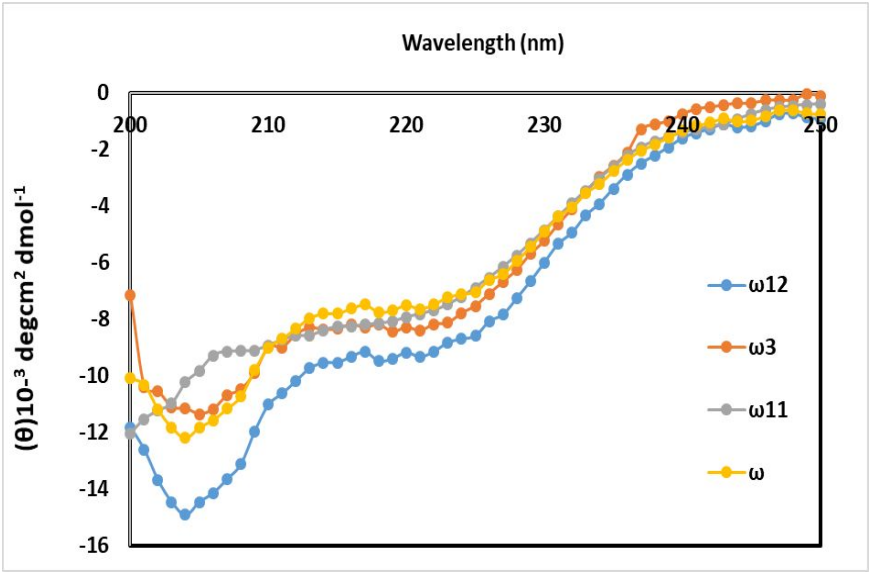


Figure S5. CD curve of all the mutants. (A) CD curves of the mutant showing lethal phenotype and (B) CD curves of mutant which had no change in phenotype. Lethal phenotype indicates that when their expression was induced, cells were dying.

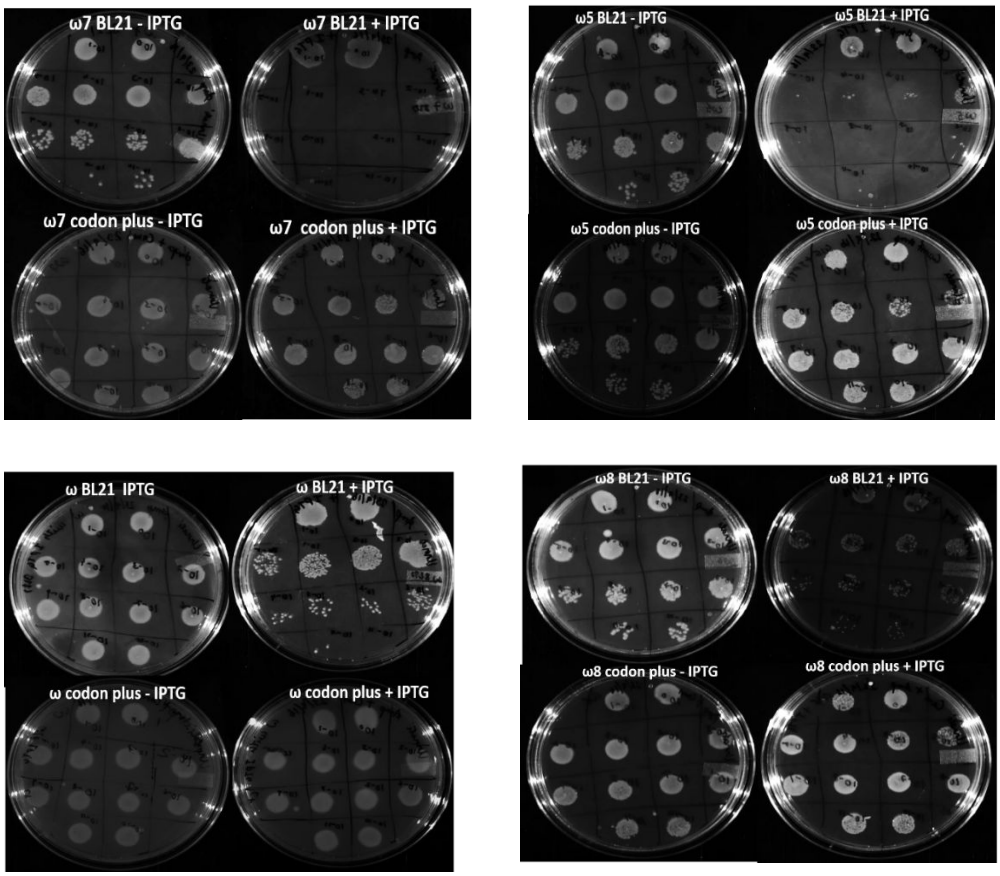
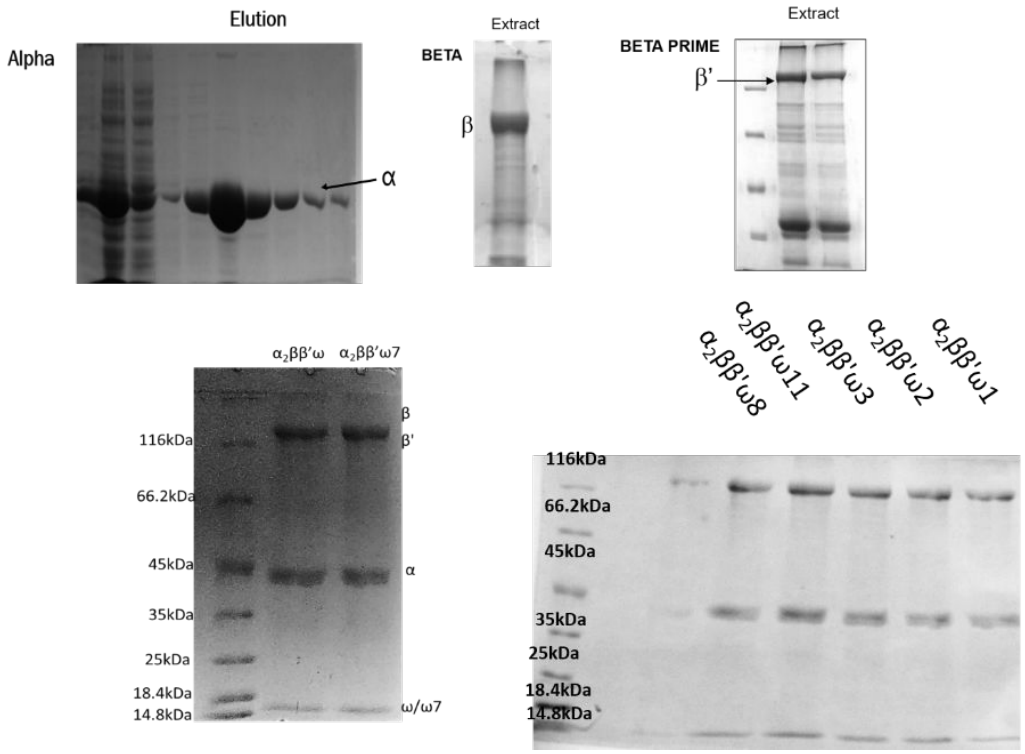


Figure S6. CFU assay to follow rescue phenotype for mutants.

BL21 and codon plus strain transformed cells were plated on LB agar plates with and without IPTG (Inducer). It could be seen that lethal phenotype of structured mutant protein was rescued in codon plus background.

(A)


Elution
Alpha
α
Extract
BETA
β
Extract
BETA PRIME
β'
α2ββ'ω8
α2ββ'ω11
α2ββ'ω3
α2ββ'ω2
α2ββ'ω1
α2ββ'ω
α2ββ'ω7
116kDa
66.2kDa
45kDa
35kDa
25kDa
18.4kDa
14.8kDa
β
β'
α
ω/ω7
116kDa
66.2kDa
45kDa
35kDa
25kDa
18.4kDa
14.8kDa

(B)

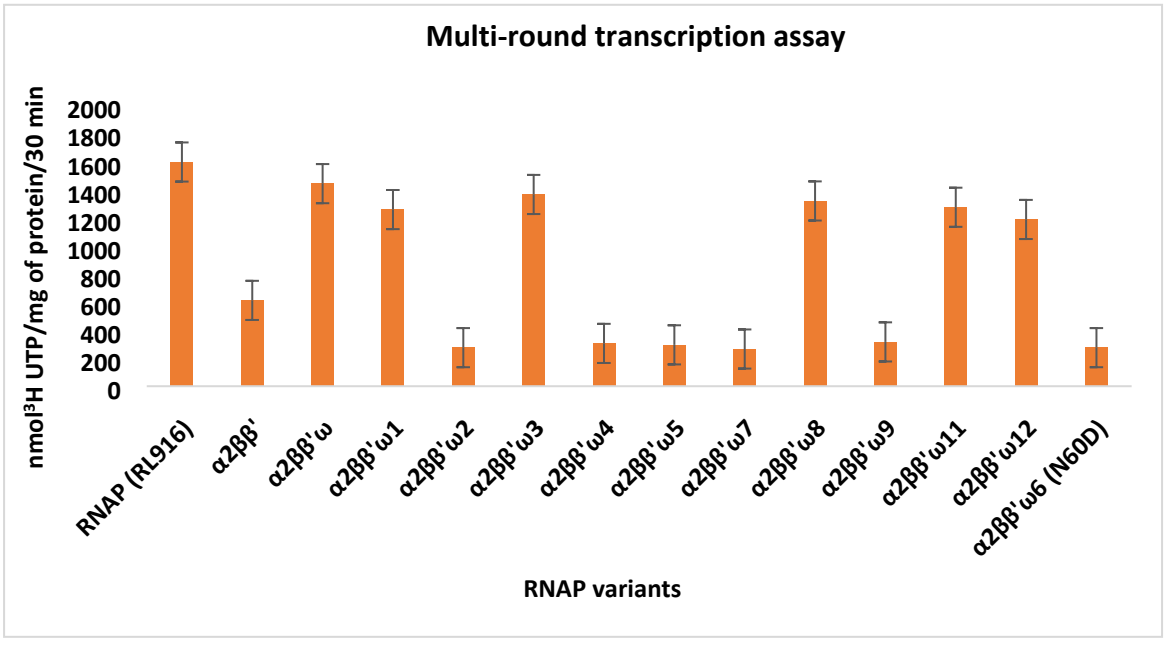
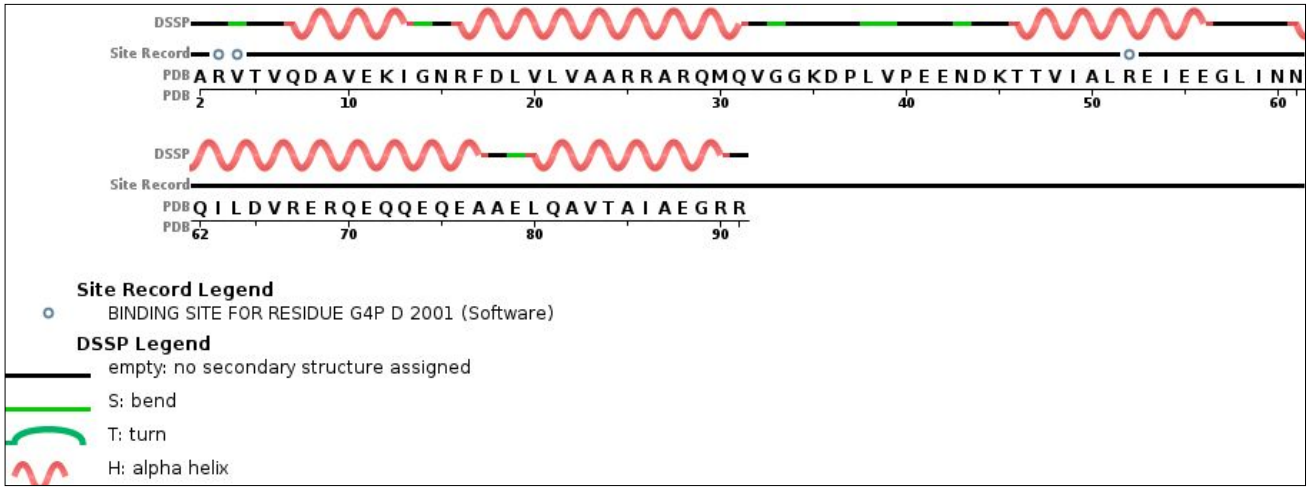

Multi-round transcription assay
nmol³H UTP/mg of protein/30 min
2000
1800
1600
1400
1200
1000
800
600
400
200
0
RNAP (RL916)
α2ββ'
α2ββ'ω
α2ββ'ω1
α2ββ'ω2
α2ββ'ω3
α2ββ'ω4
α2ββ'ω5
α2ββ'ω7
α2ββ'ω8
α2ββ'ω9
α2ββ'ω11
α2ββ'ω12
α2ββ'ω6 (N60D)
RNAP variants

Figure S7. *In vitro* Reconstitution and Transcription assay. *(A)* Individual purified subunit and reconstituted RNA polymerase *(B)* Multi-round transcription assay. RNA polymerase reconstituted with ω silent mutants which are structured were transcriptionally compromised. RNA polymerase reconstituted with structured mutants showed five times less activity as compared to that reconstituted with wild type protein

(A)



(B)

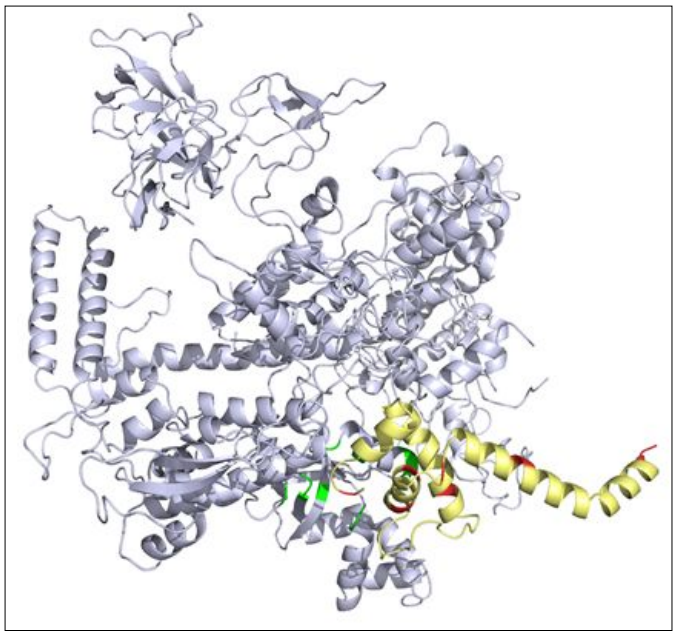


Figure S8. Secondary structure of free form and bound Omega (A). Secondary structure in bound form of ω from RNA polymerase crystal structure (PDB id 4JKR) (B) Interaction between ω and β' at the mutation sites. ω latches β' and helps in assembly of RNA polymerase [15].

Supplementary Table S1 . List of primers used to generate silent mutants of ω

Primers	Sequence
ω_1F	5' CATATGGCAAGGGTAACTGTTCAGGACGCTGTAGAGAAAATT 3'
ω_1R	5' ACGGTTACCAATTTTCTCTACAGCGTCCTGAACAGTTACCCTTGCCAT 3'
ω_2F	5' GTTCGCGAAAGGCAGGAACAGCAAGAGCAGGAAGCCGCTGAA 3'
ω_2R	5' CTGTTCCTGCCTTTCGCGAACGTCGAGGATCTGGTTGTTGAT 3'
ω_5F	5' AACAACCAGATACTCGACGTTCGCGAACGCCAGGAACAGCAA 3'
ω_5R	5' AACGTCGAGTATCTGGTTGTTGATCAGACCTTCTTCGAT 3'
ω_7F	5' ACCACTGTAATAGCGCTGCGCGAAATAGAAGAAGGTCTGATCAACAACCAG 3'
ω_7R	5' CAGACCTTCTTCTATTTCGCGCAGCGCTATTACAGTGGTTTTATCGTTTTCTTCCGG 3'
ω_8F	5' ACCACTGTAATAGCGCTGCGCGAAATCGAAGAAGGTCTG 3'
ω_8R	5'GCGCAGCGCTATTACAGTGGTTTTATCGTTTTCTTCCGGTAC 3'
ω_{10}F	5' ATAGCGCTGCGCGAAATAGAAGAAGGTCTGATAAACAACCAGATCCTC 3'

ω_{10}R	5’ CTGGTTGTTTATCAGACCTTCTTCTATTTCGCGCAGCGCTATTACAGTGGTTTTATC 3’
ω_{11}F	5’ ACC GCT ATT GCT GAA GGT AGA AGA TAA AAG CTT
ω_{11}R	5’ AAGCTTTTATCTTCTACCTTCAGCAATAGCGGTAACGGCTTGTAATTCAGC 3’

Supplementary Table S2: List of strains used for the study

Strains/Plasmid	Description	Source/Reference
E. coli DH5α	*E. coli* host strain for DNA cloning	Laboratory stock
E. coli BL21 (DE3)	*E. coli* host strain for protein expression	Laboratory stock
E. coli codon plus	*E. coli* host strain for protein expression	Laboratory stock
pET21b	*E. coli* expression vector (Amp^r)	Novagen
pET28b	*E. coli* expression vector (Kan^r)	Novagen
E. coli RL916	*E. coli rpoC* chromosomally His-tagged	A kind gift from Robert Landick
pET-ω	*rpoZ* cloned in pET28b vector	Generated for this work